Table 5 740 AT #909 Amino Acid sequence comparison

Clone	Score	Pvalue	Identities	Positives
Human L19 ribosomal protein P14118	556 (255.8 bits)	6.50E-72	101/156 (69%)	124/146 (84%)
Mouse L19 ribosomal protein P22908	556 (255.8 bits)	6.50E-72	101/146 (69%)	124/146 (84%)
A. thaliana L19 ribosomal protein P49693	542 (249.3 bits)	8.90E-70	105/118 (88%)	109/118 (92%)
D. discoideum L19 ribosomal protein P14329	537 (247.0 bits)	2.90E-69	99/146 (67%)	121/146 (82%)
D. melanogaster L19 ribosomal protein P36241	530 (243.8 bits)	2.50E-68	99/146 (67%)	118/146 (80%)
S. pombe L19 ribosomal protein O42699	526 (242.0 bits)	9.70E-68	98/140 (70%)	116/140 (82%)
C. elegans L19 ribosomal protein O02639	503 (231.4 bits)	1.40E-64	91/139 (65%)	116/139 (83%)

Replace the paragraphs beginning at page 72, line 1 and ending at page 74, line 9 with the following paragraphs:

EXAMPLE 22

Novel requirements for production of infectious viral vector *in vitro* derived RNA transcripts.

This example demonstrates the production of highly infectious viral vector transcripts containing 5' nucleotides with reference to the virus vector. Construction of a library of subgenomic cDNA clones of TMV and BMV has been described in Dawson *et al.*, *Proc. Natl. Acad. Sci. USA* 83:1832-1836 (1986) and Ahlquist *et al.*, *Proc. Natl. Acad. Sci. USA* 81:7066-7070 (1984). Nucleotides were added between the transcriptional start site of the promoter for *in vitro* transcription, in this case T7, and the start of the cDNA of TMV in order to maximize transcription product yield and possibly obviate the need to cap virus transcripts to insure infectivity. The relevant sequence is the T7 promoter ...TATAG^TATTTT (SEQ ID NO: 61) where the ^ indicates the base preceding is the start site for transcription and the bold letter is the first base of the TMV cDNA. Three approaches were taken: 1) addition of G, GG or GGG between the start site of transcription and the TMV cDNA (... TATAGGTATTT, SEQ ID NO: 62, and associated sequences); 2) addition of G and a random base (GN or N2) or a G and two random bases (GNN or N3) between the start site of transcription and the TMV cDNA (...TATAGNTATTT, SEQ ID NO: 63, and associated sequences), and the addition of a GT and a single random base between the start site of transcription and the TMV cDNA (...TATAGTNGTATTT, SEQ ID NO: 64 and associated sequences). The use of random bases was based on the hypothesis that a particular base may be best suited for an additional nucleotide attached to the cDNA, since it will be complementary to the normal nontemplated base incorporated at the 3'-end of the TMV (-) strand RNA. This allows for more ready mis-initiation and restoration of wild type sequence. The GTN would allow the mimicking of two potential sites for initiation, the added and the native sequence, and facilitate more ready mis-initiation of transcription *in vivo* to restore the native TMV cDNA sequence. Approaches included cloning GFP expressing TMV vector sequences into vectors containing extra G, GG or GGG bases using standard molecular biology techniques. Likewise, full length PCR of TMV

expression clone 1056 was done to add N2, N3 and GTN bases between the T7 promoter and the TMV cDNA. Subsequently, these PCR products were cloned into pUC based vectors. Capped and uncapped transcripts were made *in vitro* and inoculated to tobacco protoplasts or *Nicotiana benthamiana* plants, wild type and 30k expressing transgenics. The results are that an extra G, ... TATAGGTATTTT, SEQ ID NO: 65, or a GTC, ... TATAGTCGTATTTT, SEQ ID NO: 66, were found to be well tolerated as additional 5' nucleotides on the 5' of TMV vector RNA transcripts and were quite infectious on both plant types and protoplasts as capped or non-capped transcripts. Other sequences may be screened to find other options. Clearly, infectious transcripts may be derived with extra 5' nucleotides.

Other derivatives based on the putative mechanistic function of the GTN strategy that yielded the GTC functional vector are to use multiple GTN motifs preceeding the 5' most nt of the virus cDNA or the duplication of larger regions of the 5'-end of the TMV genome. For example: TATA^GTNGTNGTATT, SEQ ID NO: 67, or TATA^GTNGTNGTNGTNGTATT, SEQ ID NO: 68, or TATA^GTATTTGTATTT, SEQ ID NO: 69. In this manner the replication mediated repair mechanism may be potentiated by the use of multiple recognition sequences at the 5'-end of transcribed RNA. The replicated progeny may exhibit the results of reversion events that would yield the wild type virus 5' virus sequence, but may include portions or entire sets of introduced additional base sequences. This strategy can be applied to a range of RNA viruses or RNA viral vectors of various genetic arrangements derived from wild type virus genome. This would require the use of sequences particular to that of the virus used as a vector.

EXAMPLE 23

Infectivity of uncapped transcripts.

Two TMV-based virus expression vectors were initially used in these studies pBTI 1056 which contains the T7 promoter followed directly by the virus cDNA sequence (...TATAGTATT...), and pBTI SBS60-29 which contains the T7 promoter (underlined) followed by an extra guanine residue then the virus cDNA sequence (...TATAGGTATT, SEQ ID NO: 70...). Both expression vectors express the cycle 3

shuffled green fluorescent protein (GFPc3) in localized infection sites and systemically infected tissue of infected plants. Transcriptions of each plasmid were carried out in the absence of cap analogue (uncapped) or in the presence of 8-fold greater concentration of RNA cap analogue than rGTP (capped). Transcriptions were mixed with abrasive and inoculated on expanded older leaves of a wild type *Nicotiana benthamiana* (Nb) plant and a Nb plant expressing a TMV U1 30k movement protein transgene (Nb 30K). Four days post inoculation (dpi), long wave UV light was used to judge the number of infection sites on the inoculated leaves of the plants. Systemic, noninoculated tissues, were monitored from 4 dpi on for appearance of systemic infection indicating vascular movement of the inoculated virus. Table 6 shows data from one representative experiment.

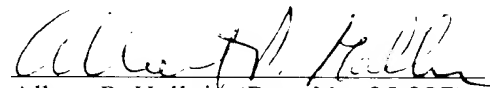**Replace the paragraph beginning at page 75, line 20 with the following paragraph:**

Data concerning cap dependent transcription of pBTI1056 GTN#28.

TMV-based virus expression vector pBTI 1056 GTN#28 which contains the T7 promoter (underlined) followed GTC bases (bold) then the virus cDNA sequence (...TATAGTCGTATT, SEQ ID NO: 71). This expression vector expresses the cycle 3 shuffled green fluorescent protein (GFPc3) in localized infection sites and systemically infected tissue of infected plants. This vector was transcribed *in vitro* in the presence (capped) and absence (uncapped) of cap analogue. Transcriptions were mixed with abrasive and inoculated on expanded older leaves of a wild type Nicotiana benthamiana (Nb) plant and a Nb plant expressing a TMV U1 30k movement protein transgene (Nb 30K). Four days post inoculation (dpi) long wave UV light was used to judge the number of infection sites on the inoculated leaves of the plants. Systemic, non-inoculated tissues, were monitored from 4 dpi on for appearance of systemic infection indicating vascular movement of the inoculated virus. Table 7 shows data from two representative experiments at 11 dpi.

No new matter is added in the amendment. The Examiner is respectfully requested to enter the amendment.

Respectfully submitted,

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